

THE CLAIMS

We Claim:

5 1. A method of detecting a target polynucleotide which comprises the steps of:

(a) contacting a target polynucleotide having a first portion and a second portion immediately contiguous to one another with:

10 (i) an invader oligonucleotide, at least a part of which is capable of specifically hybridizing to the first portion of the target polynucleotide;

(ii) a probe oligonucleotide comprising a first region that is capable of specifically hybridizing to the second portion of the target polynucleotide and a flap region located adjacent to the first region; and

15 (iii) a reagent that is capable of cleaving the flap region of the probe oligonucleotide when the probe oligonucleotide is hybridized to the second portion of the target polynucleotide and the invader oligonucleotide is hybridized to the first portion of the polynucleotide;

20 under conditions such that the cleaved flap region of the probe oligonucleotide and the reagent can come into contact with a reporter precursor to which the flap region of the probe oligonucleotide is capable of hybridizing to form a complex that can be cleaved by the reagent to provide a reporter capable of being detected;

(b) detecting the reporter to provide a signal; and

(c) determining whether the signal exhibits a specific behavior as a function of time.

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2. The method of claim 1 wherein the invader oligonucleotide comprises a first region that is capable of specifically hybridizing to the first portion of the target polynucleotide, and a flap region located adjacent to the first region.

30 3. The method of claim 2 wherein the flap region of the invader oligonucleotide is capable of specifically hybridizing to the target polynucleotide.

4. The method of claim 2 wherein flap region of the invader oligonucleotide is not capable of specifically hybridizing to the target polynucleotide.

5. The method of claim 2 wherein flap region of the invader oligonucleotide comprises a first section that is not capable of specifically hybridizing to the target polynucleotide, and a second section that is capable of specifically hybridizing to the target polynucleotide.

6. The method of claim 1 wherein the specific behavior as a function of time is non-linear.

7. The method of claim 6 wherein the specific behavior as a function of time is quadratic.

8. The method of claim 1 wherein the second portion of the target polynucleotide is located immediately 3' to the first portion of the target polynucleotide.

9. The method of one of claims 2-5 wherein the flap region of the invader oligonucleotide is located immediately 3' to the first region of the invader oligonucleotide, and the flap region of the probe is located immediately 5' to the first region of the probe.

10. The method of claim 1 wherein the signal is fluorescence or phosphorescence.

11. The method of claim 1 wherein the determination of whether the signal exhibits a specific behavior as a function of time is performed in real time.

12. The method of claim 1 wherein the determination of whether the signal exhibits a specific behavior as a function of time is performed by:
measuring the value of the signal at a plurality of times to provide a data set;

fitting the data set to a polynomial function comprising a linear term and a quadratic term; and
determining whether the coefficient of the quadratic term is greater than zero.

- 5 13. The method of claim 1 wherein the determination of whether the signal exhibits a specific behavior as a function of time is performed by:
- transforming the signal to a new domain to provide a transformed signal;
fitting the transformed signal to a first mathematical function; and
comparing the shape or behavior of the first mathematical function to the shape or
10 behavior of a linear function.